On a possible relationship between the structural peculiarities of normal and teratological fruits of Passiflora gracilis and some physico-chemical properties of their expressed juices

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We present here an account of first studies on the physical properties of the juice expressed from normal and teratological plant organs.

Materials

The plants furnishing the fruits used were vigorous and normal in growth but were transplanted from the greenhouse to the field too late to attain the largest size or to produce the maximum number of mature fruits before the oncoming of cold weather. We were, therefore, somewhat limited in amount of material, but altogether 10,929 fruits were dissected in obtaining abnormals and normals for checks. Not many more could have been worked over with the facilities available.

The fruits for which adequate samples could be secured fell into the following classes:*

(*a*) Normal fruits. Six external sutures, three placentae, no prolification. A sample of such fruits, collected as described below, served as a check for each of the samples of abnormals.

(*b*) Seven external sutures, three placentae, no prolification; 2 samples, 1, 2.

(*c*) Eight external sutures, three placentae, no prolification; 1 sample, 3.

(*d*) Eight external sutures, four placentae, no prolification; 9 samples, 4–12.

(*e*) Six external sutures, three placentae, slight and generally abortive prolification of three external carpels; 1 sample, 13.

* For a general account of prolification in *Passiflora* see a paper by J. A. Harris, Prolification of the Fruit in *Capsicum* and *Passiflora*. Ann. Rep. Missouri Bot. Gard. **17**: 133–145. 1906.

(*f*) Six external sutures, three placentae, slight and generally abortive prolification of four external carpels; 4 samples, 14–17.

(*g*) Eight external sutures, four placentae, slight and generally abortive prolification of three external carpels; 1 sample, 18.

(*h*) Eight external sutures, four placentae, slight and generally abortive prolification of four external carpels; 2 samples, 19, 20.

(*i*) Six external sutures, three placentae, large living prolification of four external carpels; 2 samples, 21, 22.

(*j*) Eight external sutures, four placentae, large living prolification of four external carpels; 1 sample, 23.

METHODS

The rarity of the abnormal fruits is a source of great difficulty in the collection of the samples. As the fruits were dissected, each abnormal was placed in a dish provided with a ground glass cover and containing bibulous paper saturated with water in order to prevent, as far as possible, any drying out of the fruits. A normal to serve as a check was at once opened and placed in a similar receptacle. These two were kept side by side until it was necessary or convenient to combine abnormalities belonging to the same type and their check fruits in a pair of larger moist chambers. As soon as a sample of any type conveniently large for the extraction of juice was secured, the collection of another general sample and check was begun.

Thus, while the different samples of abnormals came from various plants and were necessarily held for varying lengths of time, *the fruits of each sample and of the check with which it was compared were drawn in equal numbers, and at the same time, from the same individual plants and received parallel treatment in every detail.*

The juice was secured by means of a large "beef-juice" press. It was filtered clear through a dry barium filter (S. & S. No. 589), and the depression of the freezing point (Δ) determined in the well-known Beckmann apparatus. The specific gravity of the sap at 20° C. was found by weighing in a pycnometer holding 5.2405 grams of water at 20° C. The concentration of dissolved substances was determined by evaporating a measured volume (10–15 c.c.) to dryness in glass weighing bottles, first in a water oven

at the temperature of boiling water and then completing the desiccation by heating to 105° C. for several hours.*

Depression of the freezing point, Δ, specific gravity, $d(20°/20°)$, and total solids in 100 c.c. of juice, s, are the only direct determinations entered in the table. From these, osmotic pressure in atmospheres, P, and the average molecular weight of the substances dissolved in the plant sap, M, have been calculated by the formulae

$$P = 12.060\Delta - 0.021\Delta^2$$

$$M = 1890\left(\frac{\frac{s}{d}}{100 - \frac{s}{d}}\right)\Delta$$

* It may not be amiss to consider the accuracy of our numerical data. Inasmuch as we were not concerned with the *absolute* depression of the freezing point, but only with the sign and amount of variation between normal and abnormal juices, we have not taken all of the precautions which would be necessary were the *absolute* values desired. We have found that the degree of pressing the fruits has no influence on the *relative* values, providing that the normal is treated in the same manner as the abnormal. For example: a sample was pressed lightly and the freezing point of the expressed juice determined; then the residue was pressed so that a considerable amount of juice was again obtained, and this juice was added to that from the first pressing. The *absolute* values are much different but the *relative* value remains approximately the same.

	Abnormal	Normal	Difference
Light pressing.....	$\Delta = 0.677°$ C.	$\Delta = 0.603°$ C.	+ 0.074° C.
Heavy pressing....	$\Delta = 0.705°$ C.	$\Delta = 0.628°$ C.	+ 0.077° C.

A constant *relative* value is also obtained when large samples are divided into two portions and the juice expressed from the second portion after an interval of four days, showing that holding the fruits for several days does not affect the *relative* values. The *absolute* and *relative* values of a pair of samples treated in this manner follow:

	Abnormal	Normal	Difference
First value........	$\Delta = 0.587°$ C.	$\Delta = 0.618°$ C.	− 0.031° C.
Four days later....	$\Delta = 0.677°$ C.	$\Delta = 0.713°$ C.	− 0.036° C.

We have not corrected the depression of the freezing point for the amount of supercooling before freezing, for the same reason. That is, we are concerned only with a *relative* value. We have had occasion in several instances to repeat a determination, sometimes as much as twenty-four hours later, on the same sample of juice, and in only one instance did the repetition vary from the original determination by as much as 0.010° and in no instance was the sign of the difference between a sample and its check changed.

The arguments in this paper are in all cases based upon the comparison of the samples of abnormal fruits with their checks. The differences averaged and discussed in the text are in all cases taken as abnormal *less* control, the sign of the difference being positive when there is a greater depression of freezing point, higher osmotic pressure, or higher average molecular weight, in the abnormal fruits.

ANALYSIS OF DATA

The large table gives the essential constants for the various lots of fruits. The section to the left contains the data for the samples of abnormal fruits, that to the right those for the checks.

Consider first the properties of the juice in fruits differing only in the structure of the wall.

Series (*b*) and (*c*) may be regarded as transitions between the trimerous and tetramerous fruits.

In two cases Δ is higher and in one lower than in the control samples. The negative difference is only $-0.012°$ and is but twice or thrice the estimated experimental error. The mean difference in depression of the freezing point is $+0.035°$. The differences in pressure in atmospheres, P, have the same sign as the differences in depression. In one the difference is -0.144, in the other two it takes the more substantial values of $+0.638$ and $+1.143$; the mean of the three is $+0.546$. The average molecular weight is in all three cases lower in the abnormal fruits, -7.41, -4.46 and -9.02 being the values.

The tetramerous fruits (eight external sutures, four placentae, no prolification), class (*d*), are represented by nine samples, divided as follows:

	Positive differences	Negative differences	Mean value of differences
Δ	6	3	$+0.002°$ C.
M	2	7	-2.81
P	6	3	$+0.024$

Combining (*b*), (*c*) and (*d*), we have, with headings as above,

Δ	8	4	$+0.010°$ C.
M	2	10	-3.85
P	8	4	$+0.154$

TABLE I

PHYSICO-CHEMICAL CONSTANTS FOR JUICE OF NORMAL AND ABNORMAL PASSIFLORA FRUITS

Number of sample	Values for abnormal fruits					Values for normal controls.				
	Δ Depression of freezing point	$d\frac{20^\circ}{20^\circ}$ Specific gravity	s Solids in 100 c.c. of juice	M Molecular weight	P Osmotic pressure in atmospheres	Δ Depression of freezing point	$d\frac{20^\circ}{20^\circ}$ Specific gravity	s Solids in 100 c.c. of juice	M Molecular weight	P Osmotic pressure in atmospheres
1	0.503°	1.0161	3.042	115.95	6.061	0.515°	1.0169	3.349	124.97	6.205
2	0.593°	1.0191	3.466	112.23	7.144	0.540°	1.0173	3.282	116.69	6.506
3	0.633°	1.0195	3.600	109.26	7.626	0.568°	1.0180	3.448	116.67	6.483
4	0.405°	1.0122	2.209	104.10	4.881	0.358°	1.0114	2.162	115.35	4.315
5	0.503°	1.0163	3.065	116.86	6.061	0.428°	1.0129	2.356	105.14	5.157
6	0.525°	1.0174	2.630	95.54	6.326	0.513°	1.0171	2.720	101.21	6.181
7	0.435°	1.0146	2.282	99.98	5.242	0.511°	1.0168	2.747	102.70	6.157
8	0.587°	1.0187	3.381	110.53	7.071	0.618°	1.0202	3.567	110.79	7.445
9	0.558°	1.0175	3.102	106.52	6.729	0.543°	1.0169	3.233	114.27	6.542
10	0.585°	1.0181	3.154	103.29	7.047	0.581°	1.0183	3.091	101.81	7.000
11	0.567°	1.0161	3.478	118.13	6.831	0.608°	1.0195	3.907	123.87	7.324
12	0.539°	1.0169	3.204	114.10	6.494	0.527°	1.0167	3.270	119.18	6.349
13	0.673°	1.0219	4.237	121.45	8.107	0.614°	1.0184	3.526	110.38	7.397
14	0.573°	1.0188	2.857	95.12	6.903	0.550°	1.0186	2.798	97.07	6.627
15	0.705°	1.0243	4.290	117.18	8.492	0.628°	1.0212	3.652	111.62	7.565
16	0.603°	1.0200	3.540	112.70	7.264	0.613°	1.0184	3.212	100.42	7.384
17	0.569°	1.0184	3.696	125.10	6.855	0.554°	1.0183	3.523	122.26	6.674
18	0.708°	1.0189	3.891	106.01	8.528	0.683°	1.0202	4.172	117.95	8.227
19	0.558°	1.0195	—	—	6.723	0.585°	1.0204	—	—	7.048
20	0.643°	1.0203	3.729	111.51	7.746	0.597°	1.0185	3.354	107.79	7.192
21	0.455°	1.0149	—	—	5.483	0.548°	1.0180	—	—	6.603
22	0.577°	1.0180	3.584	119.53	6.952	0.533°	1.0170	3.598	130.07	6.422
23	0.563°	1.0178	3.281	111.83	6.783	0.599°	1.0198	3.754	120.60	7.216

Or disregarding the presence of prolification and adding to these the other fruits which are abnormal (tetramerous as contrasted with trimerous) in the organization of their ovary wall (i. e. classes (*g*), (*h*), and (*j*)), we get

Δ*.	10	6	+ 0.008° C.
M.	3	12	− 4.21
P.	10	5	+ 0.151

Apparently, therefore, those fruits which are tetramerous or which show transitions between the trimerous and tetramerous condition, show a greater depression of the freezing point (and consequently a higher osmotic pressure) of their juice and a lower average molecular weight† than the trimerous ones. But the differences are so very slight, and the difficulties and sources of possible error are so many, that further studies will be required to put this conclusion on a sound basis.

Turn now from the classification of the fruits according to the characteristics of the ovary wall to a consideration of the question of prolification. Here classes (*b*), (*c*), (*d*) may be left entirely out of account.

First dividing the fruits that have prolifications into the classes, trimerous and tetramerous, with respect to the characteristics of the ovary wall, without regard to the size or the structure of the included body, we have the following:

For trimerous fruits (classes (*e*), (*f*) and (*i*)),

	Positive differences	Negative differences	Mean value of differences
Δ‡.	5	2	+ 0.016° C.
M.	4	2	+ 3.21
P.	5	1	+ 0.417

For tetramerous fruits (classes (*g*), (*h*) and (*j*)),

* Depression of freezing point is available in one series in which the sample was lost before the total solids had been determined. Hence *M* and *P* are omitted.

† The average molecular weight does not necessarily take the same direction as the difference of the freezing points would indicate, inasmuch as colloids do not affect Δ but do influence the amount of total solids and through this the average molecular weight.

‡ The depression of the freezing point is available for one case for which *M* and *P* were not calculated, for the reason noted above.

Δ*	2	2	+ 0.002° C.
M	1	2	− 5.66
P	2	1	+ 0.141

On combining trimerous and tetramerous (as above) without regard to the character of the proliferous body we have:

Δ†	7	4	+ 0.011° C.
M	5	4	+ 0.25
P	7	2	+ 0.325

Apparently the proliferous fruits tend to show a greater depression of the freezing point and a higher osmotic pressure in their expressed juices than the normal checks with which they were compared. This is true for trimerous and tetramerous fruits alone and for the two classes taken together. Considering the fruits merely as normal and abnormal, we note that these results are in good agreement with those for fruits abnormal only in respect to the structure of the wall. For the trimerous fruits the results for *M* are, however, not in accord with those for fruits abnormal with respect to the wall only, *M* being higher in the abnormals than in the controls. For the tetramerous fruits the average molecular weight is again lower than in the controls. Taking both trimerous and tetramerous fruits (with prolifications) together, we find practically no difference between the average molecular weight of the fruits containing supernumerary carpels and that of their controls.

Let us now simply classify the fruits as normal and abnormal and compare the 23 samples abnormal in some character with their controls, which are normal in all regards. We have:

	Positive difference	Negative difference	Mean value of differences
Δ	15	8	+ 0.0107° C.
M	7	14	− 2.091
P	15	6	+ 0.2275

These results emphasize the conclusions drawn from the individual classes of fruits.

* Available for one case where *M* and *P* were not calculated.

† Available for two cases where *M* and *P* were not calculated.

DISCUSSION AND CONCLUSIONS

From the determination of the depression of the freezing point, the specific gravity, and the total solids in the expressed juice of 23 samples of abnormal fruits of *Passiflora gracilis* and a like number of controls, we are led to the following conclusions:

Our experiments indicate that the juice of abnormal fruits has a higher osmotic pressure (greater depression of the freezing point) than that of normals. This is true whether the abnormality be a meristic variation in the fruit wall—i. e. an increase in the number of external sutures or of the number of placentae over the normal condition—or the production of an entirely new structure in the form of an included whorl or whorls of accessory carpels springing from the floor of the fruit (prolification of the fruit).*

The average molecular weight of the substances in solution in the plant sap is, apparently, lower in the abnormal fruits, but this is less consistently true for the various classes of structural aberrations recognized.

While the findings are fairly consistent throughout, it must be remembered that the problem is surrounded with many difficulties. We have no desire to be dogmatic concerning these conclusions, realizing that a wider series of material than we could possibly obtain is desirable,† and that many questions remain to be investigated.‡ Furthermore, it is clear that the whole problem of the nature of the relationship between the structure of the fruits and the properties of the juice remains to be worked out. We only claim to have demonstrated that the physico-chemical properties of the plant sap deserve consideration as a first step in the analysis of the factors involved in morphological variations of the fruit.

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* In a forthcoming memoir on the morphology of normal and teratological fruits of *Passiflora gracilis* one of us will show that the occurrence of prolification is to some degree correlated with abnormalities of the ovary wall.

† We hope another year to obtain a strain of plants showing a higher percentage of abnormalities or at least to obtain far larger series of dissections.

‡ In particular it will be of great interest to work out in detail the relationship between the properties of the juice of the ovary wall and that of the contained carpels in the case of fruits showing prolifications. Some beginning has been made on this problem, but as yet our data are too few to justify the discussion of this and several other points.